Hindawi
Journal of Nanomaterials
Volume 2020, Article ID 2971827, 9 pages
https://doi.org/10.1155/2020/2971827

Research Article

In Vitro Toxic Effect of Biomaterials Coated with Silver Tungstate or Silver Molybdate Microcrystals

Claudia Viviane Guimarães Pellissari,[1] Carlos Eduardo Vergani,[1] Elson Longo,[2] Ana Claudia Pavarina,[1] Paula Volpato Sanitá,[1] Walter Luiz Siqueira,[3] and Janaina Habib Jorge[1]

[1]*Araraquara Dental School, São Paulo State University (UNESP), Araraquara, Brazil*
[2]*Federal University of São Carlos (UFSCar), São Carlos, Brazil*
[3]*University of Saskatchewan, Saskatoon, Canada*

Correspondence should be addressed to Janaina Habib Jorge; habib.jorge@unesp.br

Received 15 July 2019; Accepted 2 December 2019; Published 28 January 2020

Academic Editor: Giuseppe Compagnini

Purpose. This study evaluated the cytotoxicity of antimicrobial silver tungstate (Ag_2WO_4) or silver molybdate (Ag_2MoO_4) microcrystals coating biomaterials. *Materials and Methods*. The coating procedure was performed onto titanium, zirconia, and acrylic resin specimens. Eluates of the coated specimens were obtained, which were used for cytotoxicity analyses, including Alamar Blue, MTT, and CytoTox-ONE tests. Data were analyzed using two-way ANOVA, followed by the Tukey test ($\alpha = 0.05$). The results of each experimental group were also compared to those of the control of living cells, taken as 100% cell viability. *Results*. In general, it was observed that the percentage of living cells from all biomaterials coated with both microcrystals was statistically different compared to the ones from the uncoated sample groups, except for the results from MTT of specimens of Ti coated with α-Ag_2MoO_4. All uncoated biomaterials were classified as noncytotoxic by the three assays used in the present study. It was observed that the microcrystals in solution were strongly cytotoxic, with death of almost 100% of cells, from the analysis of the results of the Alamar Blue assay. *Conclusion*. The most biomaterials coated with both microcrystals showed some degree of cytotoxicity in the different assays. The results described herein should be seen as an alert to the use of microcrystals, which can expose patients to health risks.

1. Introduction

Several therapies have been proposed to prevent and treat microbial infections, including those caused by biofilms. In general, the microbial biofilm formation is a multistep growth process involving pretreatment of the substrate by the formation of a layer called conditioning film, cell attachment, cell colonization, and extracellular matrix formation. Moreover, the biofilm formation can result in tolerance of microorganisms to high concentrations of various antimicrobials. This fact has an important clinical relevance because, even in the presence of treatment modalities, resistant biofilms can cause chronic infections [1]. Therefore, instead of just treating, effective therapies to prevent biofilm formation on surfaces of implanted and restorative materials are considered an essential measure against biofilm-dependent diseases.

Surfaces with antimicrobial properties are highly desirable in applications requiring a protective barrier against infection. In this context, coating surfaces with nanoparticles or microcrystals have been adopted [2–6]. In medicine and dentistry, different biomaterials, such as polymethylmethacrylate, ceramics, and titanium, could be coated with nanoparticles to improve their antimicrobial properties, especially in hindering adhesion and proliferation of microorganisms [7–12]. Silver nanoparticles (AgNPs) have been

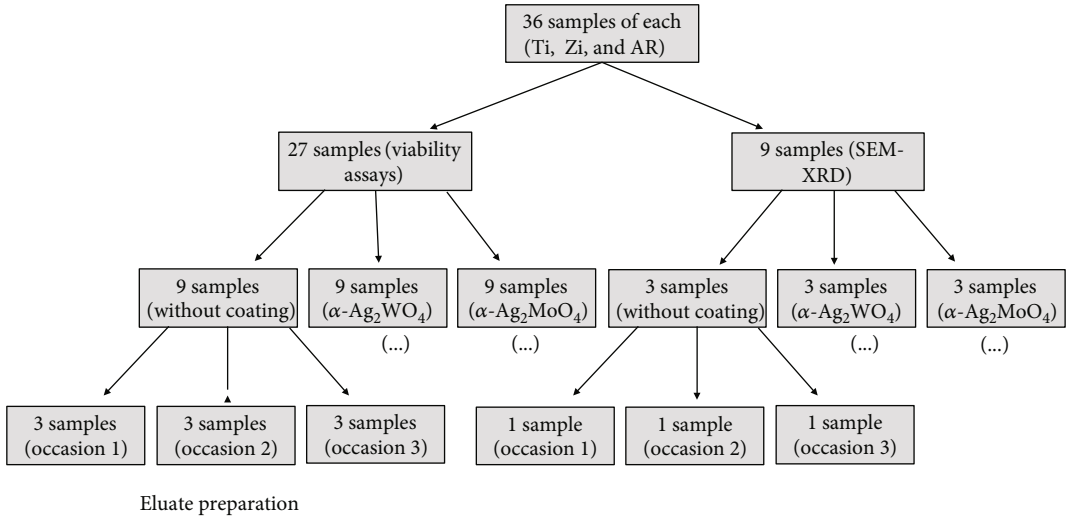

Figure 1: Experimental group distribution.

shown to have significant antimicrobial activity against planktonic cells and biofilms of *Candida glabrata*, *Candida tropicalis*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus* (MRSA) [13–17]. Recent studies have shown the antimicrobial effect of Ag as a microcrystal [18–21]. The ability of Ag_2WO_4 in fighting *Candida albicans* is related to the imperfect and crystalline patterns of atom arrangements in its orthorhombic structure and good photocatalytic capacity under visible light [21]. Also, Ag particles can increase the interaction and penetration into the cell membrane and, consequently, their antimicrobial activity [13, 16, 17].

The same mechanisms involved in killing microorganisms via Ag particles may cause human cell death, limiting its clinical application. Studies have revealed that the consequences of using Ag nanoparticles include potential changes in the cognitive, sensory, and motor functions, which result in brain and liver damage [22, 23]. Thus, it is paramount to perform biocompatibility studies to elucidate this issue. In spite of the expanding application of microcrystals within dentistry as antimicrobial agents, the biological responses of these new treatments have been insufficiently evaluated [22–24]. Thus, the aim of this study was to evaluate the cytotoxicity of extracts from silver tungstate (Ag_2WO_4) or silver molybdate (Ag_2MoO_4) coating biomaterials (titanium, zircon, and acrylic resin) through the human cell culture method.

2. Materials and Methods

The synthesis and characterization by X-ray diffraction (XRD) patterns of Ag_2WO_4 and Ag_2MoO_4 microcrystals were performed in the Functional Materials Development Center, directed by Professor Elson Longo, and published in the previous study [25]. In the same way, shapes and sizes of the Ag_2WO_4 and Ag_2MoO_4 microcrystals were observed by field emission scanning electron microscopy (FE-SEM), using the same methodology described by Santana et al. [25]

Thirty-six discs of each titanium (Ti), zircon (Zi), and acrylic resin (AR) were prepared (8 mm in diameter and 2 mm in thickness) and distributed in the experimental groups as described in Figure 1. Pure titanium alloy discs were donated by Conexão Sistemas de Prótese (Arujá, SP, Brazil). Zirconia Lava™ (3M Espe, Saint Paul, MN, USA) discs were prepared using the Ceramill Motion software system. Acrylic resin specimens were prepared in a metal matrix for discs with the resin denture base Vipi Wave (VIPI Indústria e Comércio Exportação e Importação de Produtos Odontológicos Ltda, Pirassununga, SP, Brazil), according to the manufacturer's instructions. All AR samples were left in distilled water at 37°C for 48 hours to release the residual monomer [26–29].

The coating procedure of microcrystals on discs was performed using a precipitation technique. Suspensions of Ag_2WO_4 or Ag_2MoO_4 microcrystals at a concentration of 1 mg/mL each in isopropyl alcohol were subjected to ultrasound for 20 minutes. Then, 5 μL of each suspension was dripped onto the upper surface of the discs. After 20-minute drying, the dripping was repeated 5 times. After deposition of all layers, the samples were heat treated at 250°C (Ti and Zi) or 100°C (AR) for 2 hours. Scanning electron microscopy (SEM) analysis was carried out to demonstrate the deposition of microcrystals onto disc surfaces.

To analyze the cytotoxic effect, the coated biomaterials were subjected to procedures to obtain eluates of soluble substances. Specimens were ultrasonically cleansed in distilled water for 20 minutes and kept for 20 minutes under ultraviolet light to prevent microbial contamination [26–29]. Then, three specimens of each biomaterial (coated or not with the different microcrystals) were placed in polypropylene tubes with 2.5 mL of DMEM culture medium and incubated (37°C/24 hours) [26–29]. Another tube containing only 2.5 mL of culture medium was stored under the same conditions, thus serving as a negative control group. Human keratinocytes (HaCaT cell line 0341) were acquired from the Cell Bank of Rio de Janeiro (Rio de Janeiro, RJ, Brazil). HaCaT cells were grown in plastic

bottles of 75 cm^2 with DMEM culture medium containing 10% of fetal bovine serum (FBS), with 2.0 mmol/L L-glutamine, 10,000 $\mu g\,mL^{-1}$ penicillin G, 10,000 $\mu g\,mL^{-1}$ streptomycin, and 25 $\mu g\,mL^{-1}$ amphotericin (Sigma-Aldrich, Saint Louis, Missouri, USA), at 5% CO_2/37°C. For maintenance culture, the cells were cultured until they reach confluence (90%), washed with phosphate buffer 1X PBS (140 mmol L^{-1} NaCl, 3.0 mmol L^{-1} KCl, 4.30 mmol L^{-1} Na_2HPO_4, and 1.4 mmol L^{-1} KH_2PO_4), removed with trypsin solution (0.05%, containing 0.53 mmol L^{-1} EDTA), and then subjected to centrifugation (400 × g/5 min). Next, the cells were resuspended in DMEM culture medium and counted in a Neubauer chamber. The cells were plated at 1.5×10^4 cells/well in sterile 96-well plates and incubated (5% CO_2/37°C). After 24 hours, the cells were exposed to eluates and incubated for another 24 hours. The exposed cells were subjected to three cytotoxicity assays. In addition, the cell morphology was observed with an inverted optical microscope (Model 403, Optiphase, Van Nuys, CA, USA).

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was carried out to assess the cellular metabolism. The HaCaT cells exposed to eluates from coated materials were washed with 150 μL of 1X PBS. Next, 150 μL of MTT solution (5.0 mg/mL^{-1}; Sigma-Aldrich, Saint Louis, Missouri, USA) was added to each well of a 96-well microplate, followed by incubation (37°C/5% CO_2/4 hours in the dark). Then, the formazan crystals were solubilized in 75 μL of acidified 2-propanol with 0.04 N HCl. After stirring and checking the homogeneity of the solutions, absorbance reading was performed at 570 nm in Microplate Reader EZ 400 (Biochrom, Cambourne, Cambridge, UK). Cell proliferation of the keratinocytes was also assessed using the Alamar Blue® assay. The HaCaT cells exposed to eluates were washed with 150 μL of 1X PBS. Then, an aliquot of 150 μL of diluted Alamar Blue (Molecular Probes, Invitrogen Corporation, Waltham, Massachusetts, USA) solution (10% of Alamar Blue solution plus 90% of DMEM with 10% FBS) was added to each well of a 96-well microplate, followed by incubation (37°C/5% CO_2/4 hours). After this period, the contents of the wells were transferred to a sterile black 96-well plate with a flat bottom and fluorescence was read immediately using Fluoroskan Ascent FL (Thermo Fisher Scientific, Marietta, Ohio, USA), with a filter of 544 nm of emission and 590 nm of transmission. The test with the CytoTox-ONE™ reagent measures the lactate dehydrogenase (LDH) release from cells with damaged membranes. The production of fluorescent product is proportional to the amount of LDH released. The cell culture plate was removed from the CO_2 incubator, and 3 μL of lysis solution was added to each well. Then, the plate was placed for 30 minutes in an incubator at 22°C. After this period, 150 μL of the reagent CytoTox-ONE (Homogeneous Membrane Integrity Assay, Promega, Madison, WI, USA) was added to each well and the plate was incubated again at 22°C for 10 minutes in the absence of light. Then, 75 μL of "stop solution" was added to all wells, and the contents were gently homogenized and transferred to a new sterile black 96-well plate with a flat bottom. The fluorescence was read immediately using Fluoroskan Ascent FL, with a filter of 544 nm of emission and 590 nm of transmission. The assays were performed in three separated experiments.

Besides the assessment of cytotoxicity of biomaterials after coating with both microcrystals, cytotoxicity of Ag_2WO_4 and Ag_2MoO_4 microcrystals in solution was also analyzed. For the preparation of solutions, each sintered powder, after sterilization, was dispersed in sterile distilled water in Falcon tubes, with the final concentration of 1 mg/mL. To perform the cytotoxicity assay, serial dilutions, in DMEM culture medium with 10% FBS, were made to reach concentrations of 0.25 mg/mL and 0.125 mg/mL for both microcrystals. These concentrations were selected according to the minimum inhibitory concentrations (MIC) determined after previous microbiological tests [18, 19, 30]. The cells (HaCaT cell line 0341) were plated at 1.5×10^4 cells/well in sterile 96-well plates and incubated (5% CO_2/37°C). After 24 hours, the cells were exposed to microcrystals in solution and incubated for another 24 hours. Cells that were not exposed to the solution of microcrystals served as a negative control. All cells were subjected to the Alamar Blue assay, as previously described.

In the quantitative analysis, the results of the viable cells obtained on different tests (MTT, Alamar Blue, and CytoTox-ONE) were tabulated and subjected to the tests of normality (Shapiro-Wilk) and variance homogeneity (Levene) to verify the distribution of the variables. To assess the cytotoxicity of the three biomaterials coated with Ag_2WO_4 or Ag_2MO_4 microcrystals, a two-factor analysis of variance (two-way ANOVA) was applied, followed by the Tukey multiple comparison test, with 5% significance level for decision-making. In these analyses, the percentage of living cells of all the experimental groups was compared to that of the control. For the qualitative analysis, the results of each experimental group were compared with those of the control group (taken as 100% viability). The biomaterials and microcrystals used were ranked according to the cytotoxic effect [31]: noncytotoxic (inhibition less than 25% compared to the control group), slightly cytotoxic (inhibition between 25% and 50% compared to the control group), moderately cytotoxic (inhibition between 50% and 75% compared to the control group), and strongly cytotoxic (inhibition greater than 75% compared to the control group).

3. Results

Results about the synthesis and characterization by X-ray diffraction (XRD) patterns of Ag_2WO_4 and Ag_2MoO_4 microcrystals can be observed in the study reported by Santana et al. [25]. Figure 2 shows Ag_2WO_4 and Ag_2MoO_4 microcrystals on the surfaces of biomaterials: titanium (Ti), zircon (Zi), and acrylic resin (AR).

Table 1 shows the results of living cell percentages relative to control (considered 100% living cells), for all experimental conditions. It is noteworthy that for the CytoTox-ONE test, the data was subtracted from 100% to determine the number of living cells since the test determines the number of dead cells by quantification of released LDH. In general, it was observed that the percentage of living cells from all biomaterials coated with both microcrystals was statistically different compared

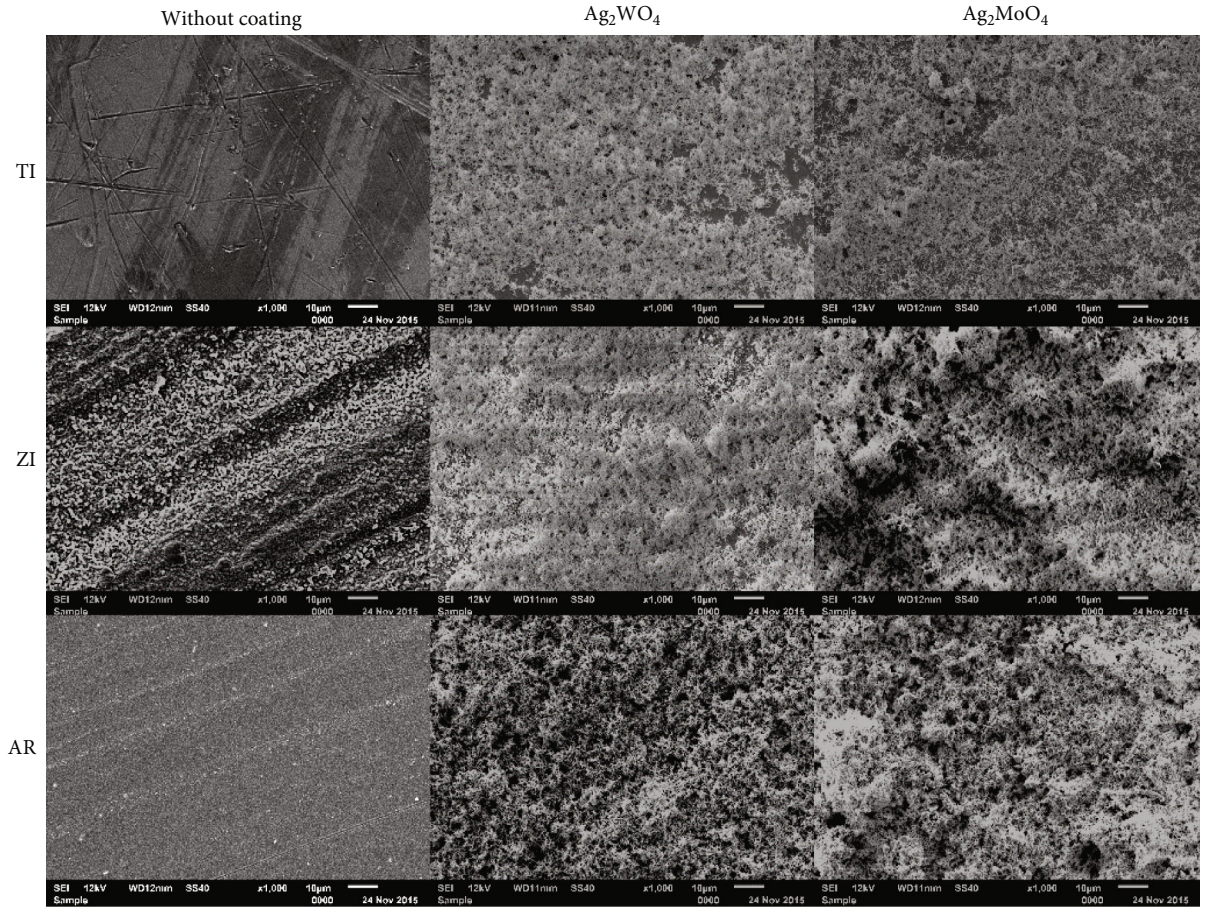

Figure 2: SEM representative images of the surface characteristics of each biomaterial before and after the deposition of the microcrystals.

Table 1: Mean (standard deviation) of living cell percentages compared to control, obtained in the different tests, according to the biomaterial and the employed coating.

Cytotoxic assay	Material	Coating		
		Ag_2WO_4	Ag_2MoO_4	Without coating
Alamar	Ti	77.3 (2.6)^{aA}	73.3 (1.8)^{aA}	96.9 (2.1)^{bB†}
	Zi	73.4 (1.2)^{aA}	69.2 (1.2)^{aA}	88.6 (1.3)^{aB}
	AR	76.7 (1.6)^{aA}	74.3 (1.0)^{aA}	88.6 (1.8)^{aB}
MTT	Ti	73.7 (2.8)^{bA}	76.3 (3.4)^{aAB}	84.2 (0.3)^{aB}
	Zi	63.7 (2.6)^{aA}	61.2 (2.8)^{bA}	85.2 (5.4)^{aB}
	AR	72.5 (3.9)^{abA}	71.0 (1.2)^{abA}	82.6 (3.1)^{aB}
CytoTox-ONE	Ti	66.8 (0.8)^{aA}	63.5 (1.8)^{bA}	86.4 (2.2)^{aB}
	Zi	63.7 (4.0)^{aB}	53.3 (2.1)^{aA}	75.2 (2.2)^{bC}
	AR	65.1 (1.6)^{aB}	59.2 (1.4)^{abA}	81.6 (1.1)^{aC}

Means followed by the same letter (uppercase in line or lowercase in the column) were not significantly different (Tukey test: $p > 0.05$). †Not significantly different from the living cell control.

to the ones from the uncoated sample groups, except for the results from MTT of specimens of Ti coated with Ag_2MoO_4. In the Alamar Blue test, for all the three materials tested, coating with both microcrystals significantly reduced ($p < 0.05$) the percentage of living cells in comparison to the uncoated groups. When the coated materials were compared, there were no significant differences among them ($p > 0.05$), regardless of the type of microcrystal used. Within the groups of uncoated biomaterials, only the Ti was considered statistically similar ($p > 0.05$) to the control of living cells (considered 100%) and different from the other groups ($p < 0.05$), which showed no significant differences between them ($p > 0.05$). The MTT assay showed that, except for the Ti specimens coated with Ag_2MoO_4, the percentage of

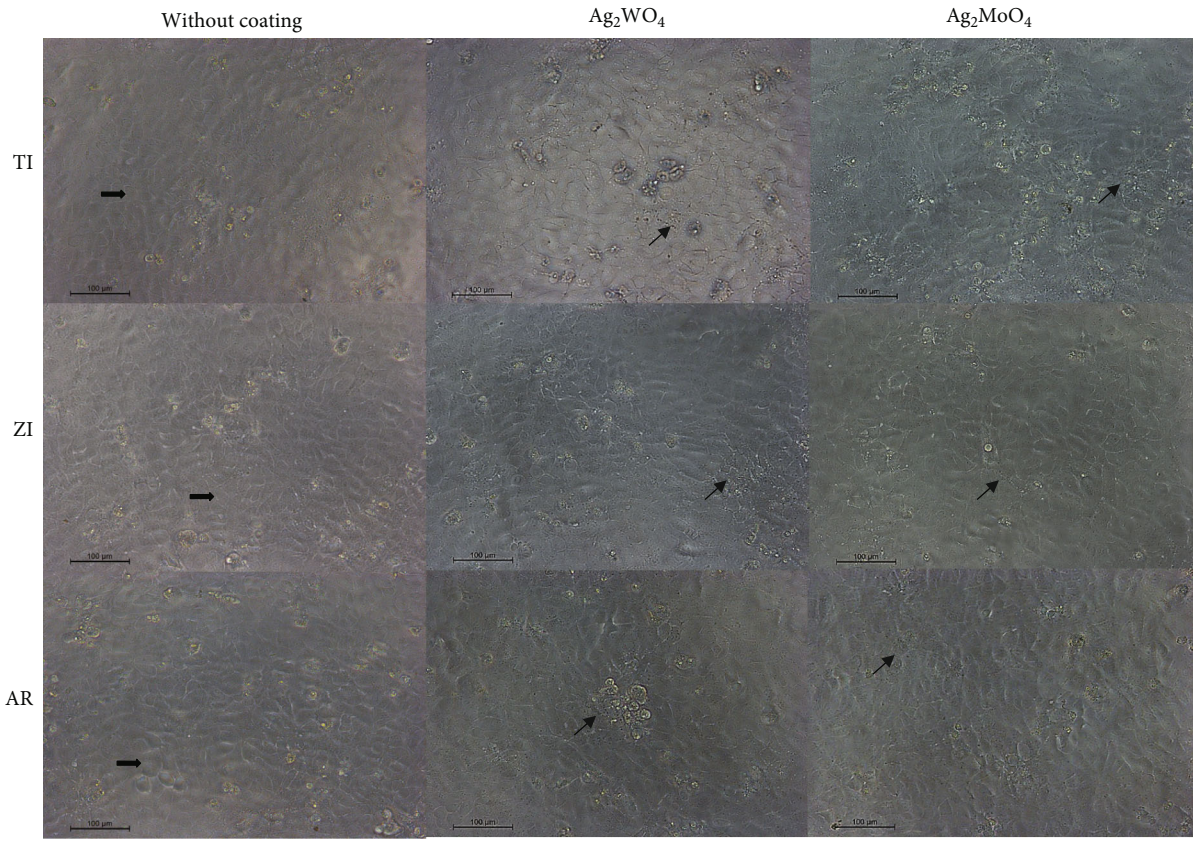

Figure 3: Cell morphology after the contact with the eluates from coated or uncoated biomaterials. The black arrows indicate the presence of particles released in the culture medium from the preparation of the extracts.

living cells from the other experimental conditions was significantly lower ($p < 0.05$) than that from the uncoated groups. When the coated materials were compared, in general, the biocompatibility of Zi was lower than that of Ti, regardless of the type of microcrystal used. There were no significant differences ($p > 0.05$) among the groups of uncoated biomaterials. All conditions tested were statistically different from the control group of living cells by this test. For the CytoTox-ONE test, the results showed that the samples of Zi and AR coated with Ag_2MoO_4 had the lowest percentages of alive cells, which were significantly different ($p < 0.05$) from the percentage obtained for the same materials coated with Ag_2WO_4. In addition, all uncoated materials demonstrated higher values ($p < 0.05$) of viable cells than the coated ones. When the biomaterials were compared, there were no significant differences ($p > 0.05$) among the Ti, Zi, and AR samples coated with Ag_2WO_4. When the specimens coated with Ag_2MoO_4 were compared, the Zi samples showed the lowest percentage of viable cells. In addition, within the uncoated material group, there were no significant differences ($p > 0.05$) between Ti and AR samples, which were statistically different from Zi samples ($p < 0.05$). All conditions tested were statistically different from the control group of living cells by this test.

All uncoated biomaterials were classified as noncytotoxic by the three assays used in the present study (Alamar Blue, MTT, and CytoTox-ONE). The same rating was given for Ti and AR specimens coated with Ag_2WO_4 by the Alamar Blue test and for Ti specimens coated with Ag_2MoO_4 by the MTT assay. The other biomaterials coated with both microcrystals were classified as slightly cytotoxic because their extracts inhibited cell growth between 25 and 50% compared to the control group.

Figure 3 illustrates the cell morphology after the contact with the eluates from biomaterials coated or uncoated (inverted optical microscope). For all groups, it was possible to observe that the cells maintained their characteristic polygonal epithelial shape, forming a confluent monolayer. Also, from Figure 3, it can also be seen that during the incubation period, Ag microcrystals were released into the culture medium.

Figure 4 shows the fluorescence results (Alamar Blue assay) of the solutions of Ag_2WO_4 and Ag_2MoO_4 microcrystals. It is possible to observe a substantial reduction of the cell viability when the experimental groups were compared to the control group. Because of this discrepant difference, statistical analysis was not necessary to establish the differences among groups. Figure 5 illustrates the percentage of cell viability for the solutions compared to the control cells (considered 100% viability). It was observed that the microcrystals in solution, in both concentrations, were strongly cytotoxic, with death of almost 100% of cells. Thus, the other tests (MTT and CytoTox-ONE) were not carried out because they were considered unnecessary.

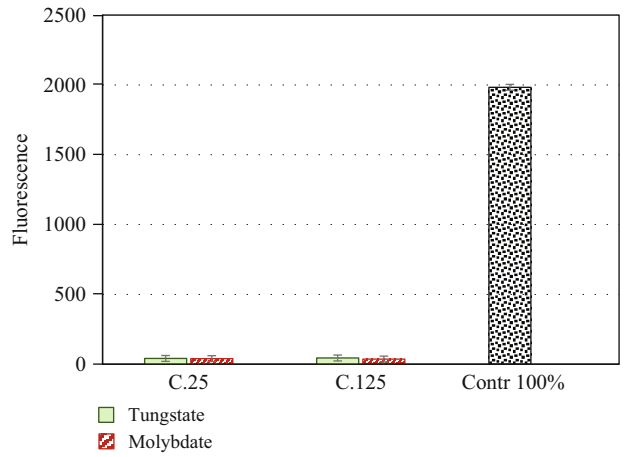

FIGURE 4: Fluorescence results from the Alamar Blue assay of Ag_2WO_4 and Ag_2MoO_4 microcrystals in solution.

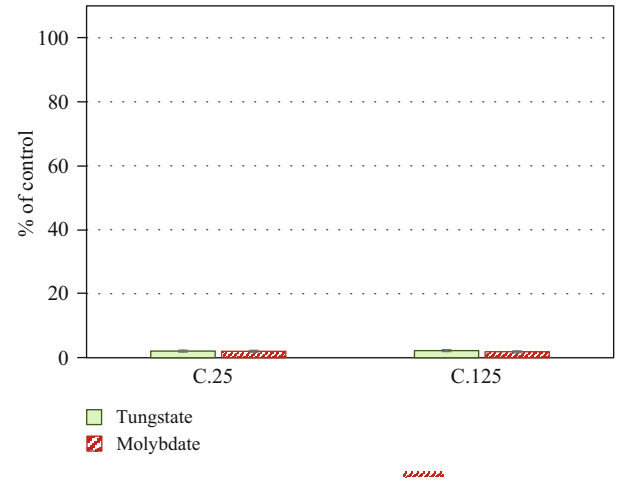

FIGURE 5: Percentage of cell viability for each group (Ag_2WO_4 and Ag_2MoO_4 microcrystals in solution) compared to the control cells (considered 100% viability).

4. Discussion

Surfaces with antimicrobial properties are highly useful in applications requiring a protective barrier against infection, and the use of nanoparticles or microcrystals represents a promising strategy, since they have the ability to inhibit the growth of microorganisms by different mechanisms [13, 32]. Despite the promising and effective antimicrobial activity [18, 19], the use of nanoparticles or microcrystals should be indicated with caution since the same death mechanisms of the microorganisms can also cause the death of human body cells. Genetic changes, systemic sclerosis, rheumatoid arthritis, lupus erythematosus, and chronic kidney disease can be caused by the exposure to nanoparticles [33–35]. Considering this information, this study evaluated the cytotoxicity of silver tungstate (Ag_2WO_4) and silver molybdate (Ag_2MoO_4), both in solution and in biomaterial coatings. For this, the cytotoxicity assays, using cultured cells and eluates, was selected because it is considered to be relatively simple, reproducible, effective, and controlled [36, 37].

MTT and Alamar Blue assays showed similar results regarding the effect of coating Ti, Zi, and AR surfaces with Ag_2WO_4 or Ag_2MoO_4. When these results were compared to the data from the CytoTox-ONE test, a divergent situation was observed, since the CytoTox-ONE test detected some differences among the experimental conditions that were not observed before. The CytoTox-ONE test seems to be more sensitive than the other ones, due the fact that, in some groups, cells with preserved mitochondrial activity showed changes in the membrane, which were detected by the release of LDH. This indicates that these methods are complementary to each other, each providing a capability lacked by the other, and thus, the results should be interpreted within the context of all data [38, 39].

The results of this study showed that Ag_2WO_4 and Ag_2MoO_4 microcrystals, in solution, decreased the cell proliferation by over 75%, when compared to the control group and, thus, were considered extremely cytotoxic. These results agree with other studies [24–40]. The reduction in cell viability could be explained, in part, due to the release of silver ions [41]. Silver nanoparticles have promising antibacterial activity for combating adhesion and biofilm formation, but their small size and high mobility require security concerns due to increased cytotoxic potential [42, 43]. Previous studies have shown that silver nanoparticles have detrimental effects on the cellular membrane [44], causing changes in its structure and, consequently, cell death. In addition, these particles can cause DNA damage and can increase reactive oxygen species, which can irreversibly impair cell functioning, also leading to cell death [45, 46]. Another way that silver nanoparticles with 20 nm in size or smaller can lead to cell death is by penetrating into the cell without endocytosis, being distributed within the cytoplasm [47]. Moreover, it has been accepted that silver nanoparticles can connect onto the cell membrane surface, causing protein denaturation and, consequently, irreversible damage to cells [48]. Nanoparticles can penetrate inside the cell and cause damage by interacting with sulfur and phosphorus compounds, such as proteins and DNA [49]. Additionally, when these nanoparticles are placed in a culture medium, they form complexes of protein and nanoparticles [50]. The formation of these complexes can also have a cytotoxic effect, due to the interaction between the protein complex layer and the cells in culture [51].

The cytotoxic effects of the Ag_2WO_4 and Ag_2MoO_4 microcrystals, in solution, could also be explained by molybdate and tungstate singly. Molybdate and tungstate are molybdenum and tungsten oxyanions, respectively, which are metallic chemical elements. Metal oxides are known for their semiconductive properties, allowing electrons to transfer between the nanomaterial and aqueous environments [52, 53]. Shape and size play an important role in determining the reactivity and the cytotoxicity of the nanoparticles [52]. The presence of metals can also participate in one-electron oxidation-reduction reactions and lead to the formation of reactive oxygen species [54], and the highly reactive free radical can interact irreversibly with organic compounds of the cells, causing collapse of the membranes and damaging DNA, RNA, and proteins of the intracellular microorganism system [24].

Currently, many studies have been published on the toxic effects of nanoparticles or microcrystals, while data on their toxicity, when used as coatings on biomaterials, are sparse. For most of the biomaterials coated with Ag_2WO_4 and Ag_2MoO_4 microcrystals, cell inhibition was observed in the order of 25% to 50%, compared to the control group, and both groups were classified as slightly cytotoxic. Although the precipitation technique has been used for coating the samples (Figure 2), the cytotoxic effect was probably due to the release of the nanoparticles from the coated biomaterial during preparation of the extracts, as shown in Figure 3. Therefore, the same effects above described for microcrystals in solution may have caused the cytotoxicity of coated biomaterials because of the release of particles in aqueous medium (eluates). Despite the inhibition of the cellular metabolism, Figure 3 illustrates that the cells maintained their characteristic polygonal epithelial shape, forming a confluent monolayer for all biomaterials coated with Ag_2WO_4 and Ag_2MoO_4 microcrystals.

The results of this in vitro study provide valuable information about the cytotoxicity of biomaterials coated with microcrystals. However, future studies are needed to understand the complex toxicity mechanisms of microcrystals, which cause cell death.

5. Conclusions

According to the results and within the limitations of this study, it can be concluded as follows:

(1) In general, the percentage of living cells from all biomaterials coated with both microcrystals was statistically different from that from the uncoated sample groups

(2) In the Alamar Blue test, for all the three materials tested, coating with both microcrystals significantly reduced the percentage of living cells in comparison to the uncoated groups

(3) In the MTT assay, for the majority of groups, the percentage of living cells from the coated biomaterials was significantly lower than that from the uncoated groups

(4) In the CytoTox-ONE test, the results showed that the samples of Zi and AR coated with Ag_2MoO_4 had the lowest percentages of alive cells, which were significantly different from the percentage obtained for the same materials coated with Ag_2WO_4

(5) In the CytoTox-ONE test, all uncoated materials demonstrated higher values of viable cells than the coated ones

(6) All uncoated biomaterials were classified as noncytotoxic by the three assays used in the present study (Alamar Blue, MTT, and CytoTox-ONE)

(7) The majority of the biomaterials coated with both microcrystals were classified as slightly cytotoxic

(8) The solutions of Ag_2WO_4 and Ag_2MoO_4 microcrystals were ranked as strongly cytotoxic

Data Availability

The statistical data used to support the findings of this study are available from corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the São Paulo Research Foundation (FAPESP) (grant numbers 2013/01844-8 and 2011/21928-6). The authors thank Professor Elson Longo, Director of the Functional Materials Development Center. The authors acknowledge the Conexão Sistemas de Próteses Ltda, for donating samples.

References

[1] R. Y. Pelgrift and A. J. Friedman, "Nanotechnology as a therapeutic tool to combat microbial resistance," *Advanced Drug Delivery Reviews*, vol. 65, no. 13-14, pp. 1803–1815, 2013.

[2] S. Areva, H. Paldan, T. Peltola, T. Närhi, M. Jokinen, and M. Lindén, "Use of sol-gel-derived titania coating for direct soft tissue attachment," *Journal of Biomedical Materials Research Part A*, vol. 70, no. 2, pp. 169–178, 2004.

[3] N. Moritz, S. Areva, J. Wolke, and T. Peltola, "TF-XRD examination of surface-reactive TiO_2 coatings produced by heat treatment and CO_2 laser treatment," *Biomaterials*, vol. 26, no. 21, pp. 4460–4467, 2005.

[4] N. Moritz, M. Jokinen, T. Peltola, S. Areva, and A. Yli-Urpo, "Local induction of calcium phosphate formation on TiO_2 coatings on titanium via surface treatment with a CO_2 laser," *Journal of Biomedical Materials Research Part A*, vol. 65, no. 1, pp. 9–16, 2003.

[5] S. Q. Sun, B. Sun, W. Zhang, and D. Wang, "Preparation and antibacterial activity of Ag–TiO_2 composite film by liquid phase deposition (LPD) method," *Bulletin of Materials Science*, vol. 31, no. 1, pp. 61–66, 2008.

[6] H. Tang, R. J. Doerksen, and G. N. Tew, "Synthesis of urea oligomers and their antibacterial activity," *Chemical Communications*, vol. 28, no. 12, pp. 1537–1539, 2005.

[7] M. T. Choy, C. Y. Tang, L. Chen, C. T. Wong, and C. P. Tsui, "In vitro and in vivo performance of bioactive Ti6Al4V/Ti-C/HA implants fabricated by a rapid microwave sintering technique," *Materials Science & Engineering. C, Materials for Biological Applications*, vol. 42, pp. 746–756, 2014.

[8] R. Depprich, M. Ommerborn, H. Zipprich et al., "Behavior of osteoblastic cells cultured on titanium and structured zirconia surfaces," *Head & Face Medicine*, vol. 4, no. 1, 2008.

[9] D. Kowalczuk, A. Przekora, and G. Ginalska, "Biological safety evaluation of the modified urinary catheter," *Materials Science & Engineering C, Materials for Biological Applications*, vol. 49, pp. 274–280, 2015.

[10] B. Möller, H. Terheyden, Y. Açil et al., "A comparison of biocompatibility and osseointegration of ceramic and titanium implants: an in vivo and in vitro study," *International Journal of Oral and Maxillofacial Surgery*, vol. 41, no. 5, pp. 638–645, 2012.

[11] C. Piconi and G. Maccauro, "Zirconia as a ceramic biomaterial," *Biomaterials*, vol. 20, no. 1, pp. 1–25, 1999.

[12] M. Huband, "Prosthetic rehabilitation," *Dermatologic Clinics*, vol. 29, no. 2, pp. 325–330, 2011.

[13] A. F. Wady, A. L. Machado, C. C. Foggi et al., "Effect of a Silver Nanoparticles Solution on _Staphylococcus aureus_ and _Candida_ spp.," *Journal of Nanomaterials*, vol. 2014, Article ID 545279, 7 pages, 2014.

[14] D. T. de Castro, M. L. C. Valente, C. H. L. da Silva et al., "Evaluation of antibiofilm and mechanical properties of new nanocomposites based on acrylic resins and silver vanadate nanoparticles," *Archives of Oral Biology*, vol. 67, pp. 46–53, 2016.

[15] S. N. Sawant, V. Selvaraj, V. Prabhawathi, and M. Doble, "Antibiofilm properties of silver and gold incorporated PU, PCLm, PC and PMMA nanocomposites under two shear conditions," *PLoS One*, vol. 8, no. 5, p. e63311, 2013.

[16] Y. Yao, Y. Ohko, Y. Sekiguchi, A. Fujishima, and Y. Kubota, "Self-sterilization using silicone catheters coated with Ag and TiO_2 nanocomposite thin film," *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 85B, no. 2, pp. 453–460, 2008.

[17] O. Choi and Z. Hu, "Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria," *Environmental Science & Technology*, vol. 42, no. 12, pp. 4583–4588, 2008.

[18] V. M. Longo, C. C. de Foggi, M. M. Ferrer et al., "Potentiated electron transference in α-Ag2WO4Microcrystals with Ag nanofilaments as microbial agent," *The Journal of Physical Chemistry A*, vol. 118, no. 31, pp. 5769–5778, 2014.

[19] M. T. Fabbro, C. C. Foggi, L. P. Santos et al., "Synthesis, antifungal evaluation and optical properties of silver molybdate microcrystals in different solvents: a combined experimental and theoretical study," *Dalton Transactions*, vol. 45, no. 26, pp. 10736–10743, 2016.

[20] M. Assis, E. Cordoncillo, R. Torres-Mendieta et al., "Towards the scale-up of the formation of nanoparticles on α-Ag_2WO_4 with bactericidal properties by femtosecond laser irradiation," *Scientific Reports*, vol. 8, no. 1, p. 1884, 2018.

[21] C. C. de Foggi, R. C. de Oliveira, M. T. Fabbro et al., "Tuning the morphological, optical, and antimicrobial properties of α-Ag2WO4Microcrystals using different solvents," *Crystal Growth & Design*, vol. 17, no. 12, pp. 6239–6246, 2017.

[22] N. El Yamani, A. R. Collins, E. Rundén-Pran et al., "In vitro genotoxicity testing of four reference metal nanomaterials, titanium dioxide, zinc oxide, cerium oxide and silver: towards reliable hazard assessment," *Mutagenesis*, vol. 32, no. 1, pp. 117–126, 2017.

[23] I. C. Chen, I. L. Hsiao, H. C. Lin, C. H. Wu, C. Y. Chuang, and Y. J. Huang, "Influence of silver and titanium dioxide nanoparticles on in vitro blood-brain barrier permeability," *Environmental Toxicology and Pharmacology*, vol. 47, pp. 108–118, 2016.

[24] N. L. Haro Chávez, E. D. de Avila, P. A. Barbugli et al., "Promising effects of silver tungstate microcrystals on fibroblast human cells and three dimensional collagen matrix models: a novel non-cytotoxic material to fight oral disease," *Colloids and Surfaces. B, Biointerfaces*, vol. 170, pp. 505–513, 2018.

[25] Y. V. De Santana, J. E. C. Gomes, L. Matos et al., "Silver molybdate and silver tungstate nanocomposites with enhanced photoluminescence," *Nanomaterials and Nanotechnology*, vol. 4, p. 22, 2014.

[26] J. H. Jorge, E. T. Giampaolo, C. E. Vergani, A. L. Machado, A. C. Pavarina, and I. Z. Carlos, "Biocompatibility of denture base acrylic resins evaluated in culture of L929 cells. Effect of polymerisation cycle and post-polymerisation treatments," *Gerodontology*, vol. 24, no. 1, pp. 52–57, 2007.

[27] J. H. Jorge, E. T. Giampaolo, C. E. Vergani, A. L. Machado, A. C. Pavarina, and I. Z. Carlos, "Effect of post-polymerization heat treatments on the cytotoxicity of two denture base acrylic resins," *Journal of Applied Oral Science*, vol. 14, no. 3, pp. 203–207, 2006.

[28] J. H. Jorge, E. T. Giampaolo, C. E. Vergani, A. L. Machado, A. C. Pavarina, and I. Z. Carlos, "Cytotoxicity of denture base resins: effect of water bath and microwave postpolymerization heat treatments," *The International Journal of Prosthodontics*, vol. 17, no. 3, pp. 340–344, 2004.

[29] J. H. Jorge, E. T. Giampaolo, C. E. Vergani, A. C. Pavarina, A. L. Machado, and I. Z. Carlos, "Effect of microwave post-polymerization treatment and of storage time in water on the cytotoxicity of denture base and reline acrylic resins," *Quintessence International*, vol. 40, no. 10, pp. e93–100, 2009.

[30] C. Foggi, R. Oliveira, A. L. Machado, C. E. Vergani, J. Andres, and E. Longo, "α-Ag_2WO_4 microcrystals synthesized in different solvents as new antibacterial agents," *Basic Research Clinical Oral Implants Research*, vol. 27, 2016.

[31] International Standard, *ISO 10993-5:2009 Biological Evaluation of Medical Devices – Part 5: Tests for In Vitro Cytotoxicity*, ISO office, 2009.

[32] Q. P. Wang, X. X. Guo, W. H. Wu, and S. X. Liu, "Preparation of fine Ag_2WO_4 antibacterial powders and its application in the sanitary ceramics," *Advances in Materials Research*, vol. 284, pp. 1321–1325, 2011.

[33] K. Donaldson and P. J. Borm, "The quartz hazard: a variable entity," *The Annals of Occupational Hygiene*, vol. 42, no. 5, pp. 287–294, 1998.

[34] X. Shi, V. Castranova, B. Halliwell, and V. Vallyathan, "Reactive oxygen species and silica-induced carcinogenesis," *Journal of Toxicology and Environmental Health. Part B, Critical Reviews*, vol. 1, no. 3, pp. 181–197, 1998.

[35] Y. Li, S. H. Doak, J. Yan et al., "Factors affecting the in vitro micronucleus assay for evaluation of nanomaterials," *Mutagenesis*, vol. 32, no. 1, pp. 151–159, 2017.

[36] J. O'Brien, I. Wilson, T. Orton, and F. Pognan, "Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity," *European Journal of Biochemistry*, vol. 267, no. 17, pp. 5421–5426, 2000.

[37] C. Wiegand and U. C. Hipler, "Methods for the measurement of cell and tissue compatibility including tissue regeneration processes," *GMS Krankenhaushygiene Interdisziplinar*, vol. 3, no. 1, 2008.

[38] A. T. H. Tang, J. Li, J. Ekstrand, and Y. Liu, "Cytotoxicity tests ofin situ polymerized resins: methodological comparisons and introduction of a tissue culture insert as a testing device," *Journal of Biomedical Materials Research*, vol. 45, no. 3, pp. 214–222, 1999.

[39] G. Ciapetti, D. Granchi, S. Stea et al., "Cytotoxicity testing of materials with limited in vivo exposure is affected by the duration of cell-material contact," *Journal of Biomedical Materials Research*, vol. 42, no. 4, pp. 485–490, 1998.

[40] J. W. Han, S. Gurunathan, J. K. Jeong et al., "Oxidative stress mediated cytotoxicity of biologically synthesized silver nanoparticles in human lung epithelial adenocarcinoma cell line," *Nanoscale Research Letters*, vol. 9, no. 1, p. 459, 2014.

[41] K. Y. Yoon, J. Hoon Byeon, J. H. Park, and J. Hwang, "Susceptibility constants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles," *Science of The Total Environment*, vol. 373, no. 2-3, pp. 572–575, 2007.

[42] H. Qin, H. Cao, Y. Zhao et al., "In vitro and in vivo anti-biofilm effects of silver nanoparticles immobilized on titanium," *Biomaterials*, vol. 35, no. 33, pp. 9114–9125, 2014.

[43] E. Longo, D. P. Volanti, V. M. Longo et al., "Toward an understanding of the growth of Ag filaments on α-Ag2WO4and their photoluminescent properties: a combined experimental and theoretical study," *The Journal of Physical Chemistry*, vol. 118, no. 2, pp. 1229–1239, 2013.

[44] C. A. Zamperini, R. S. André, V. M. Longo et al., "Antifungal applications of Ag-decorated hydroxyapatite nanoparticles," *Journal of Nanomaterials*, vol. 2013, 9 pages, 2013.

[45] Z. Ma, H. Ji, D. Tan et al., "Silver nanoparticles decorated, flexible SiO_2 nanofibers with long-term antibacterial effect as reusable wound cover," *Colloids and Surfaces*, vol. 387, no. 1-3, pp. 57–64, 2011.

[46] S. Ahlberg, M. C. Meinke, L. Werner et al., "Comparison of silver nanoparticles stored under air or argon with respect to the induction of intracellular free radicals and toxic effects toward keratinocytes," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 88, no. 3, pp. 651–657, 2014.

[47] M. Edetsberger, E. Gaubitzer, E. Valic, E. Waigmann, and G. Köhler, "Detection of nanometer-sized particles in living cells using modern fluorescence fluctuation methods," *Biochemical and Biophysical Research Communications*, vol. 332, no. 1, pp. 109–116, 2005.

[48] V. Dal Lago, L. França de Oliveira, K. de Almeida Gonçalves, J. Kobarg, and M. Borba Cardoso, "Size-selective silver nanoparticles: future of biomedical devices with enhanced bactericidal properties," *Journal of Materials Chemistry*, vol. 21, no. 33, pp. 12267–12273, 2011.

[49] A. Panacek, L. Kvítek, R. Prucek et al., "Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity," *The Journal of Physical Chemistry. B*, vol. 110, no. 33, pp. 16248–16253, 2006.

[50] T. Cedervall, I. Lynch, S. Lindman et al., "Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 7, pp. 2050–2055, 2007.

[51] C. D. Walkey, J. B. Olsen, F. Song et al., "Protein corona fingerprinting predicts the cellular interaction of gold and silver nanoparticles," *ACS Nano*, vol. 8, no. 3, pp. 2439–2455, 2014.

[52] K. M. Dunnick, M. A. Badding, D. Schwegler-Berry et al., "The effect of tungstate nanoparticles on reactive oxygen species and cytotoxicity in raw 264.7 mouse monocyte macrophage cells," *Journal of Toxicology and Environmental Health. Part A*, vol. 77, no. 20, pp. 1251–1268, 2014.

[53] N. Titenko-Holland, J. Shao, L. Zhang et al., "Studies on the genotoxicity of molybdenum salts in human cells in vitro and in mice in vivo," *Environmental and Molecular Mutagenesis*, vol. 32, no. 3, pp. 251–259, 1998.

[54] H. Meng, T. Xia, S. George, and A. E. Nel, "A predictive toxicological paradigm for the safety assessment of nanomaterials," *ACS Nano*, vol. 3, no. 7, pp. 1620–1627, 2009.